Adv. Space Res. Vol.3, No.9, pp.153-158, 1983
Printed in Great Britain. All rights reserved.

0273-1177/83 $0.00 + .50
Copyright © COSPAR

EXPERIMENTAL AND THEORETICAL ANALYSIS OF THE INFLUENCE OF GRAVITY AT THE CELLULAR LEVEL: A REVIEW

M. G. Tairbekov, G. P. Parfyonov, E. Ya. Shepelev and F. V. Sushkov

Institute of Biomedical Problems, Ministry of Health, Moscow, 123007, U.S.S.R.

ABSTRACT

The present paper is a review of the experimental investigations published in the literature and performed by the authors on space vehicles. The paper also gives an analysis of theoretical concepts concerning gravitational effects on the cell. Taking this into account, the authors put forth a hypothesis that free-living unicellular organisms are indifferent to variations in the magnitude and direction of the gravitational field.

INTRODUCTION

The evolution of the organic world, beginning with the emergence of prebiological structures and ending with present-day living creatures, is determined by their interaction with the environmental factors - physical, chemical and biological.

Gravitation or the gravity force is one of the principal physical factors that played a leading role in the generation of an environment that allowed the appearance and development of living matter, at least at the earliest stages of the evolutionary process. Geological evolution that preceded biological evolution resulted in the formation of environmental parameters required for the emergence of life on the Earth.

Gravitation was involved in the formation of a substrate, separation of the solid, liquid and gaseous phases with respect to their densities, aggregation of primitive substances to form complexes, and development of polarity. Gravitation is also involved in cyclic processes of convective transfer of matter and is responsible for the primary order in nature.

The dependence of living beings on gravity is self-evident. However, in spite of these, seemingly indubitable prerequisites, we fail to detect experimental evidence in favour of a direct involvement of gravity (or its absence, weightlessness) in these processes, when investigating fundamental biological processes associated with the cell.

The problem of adaptation to weightlessness as a space environmental factor may be considered with respect to disorders in regulatory processes at the organelle, organ and organism level as well as with respect to the pattern and rate of variations in cellular energy metabolism.

From our point of view, unicellular free-living organisms are an optimal model for studying the effects of weightlessness, since the number of their regulator complexes is small, as compared to multi-cellular organisms. However, for most cells gravitational effects are negligibly small due to their microscopic dimensions. With increase in cell size, gravitational effects become of ever increasing importance. Therefore, experimental success depends primarily on the choice of the object to be investigated and the scope of our knowledge about it.

An analysis of the results of numerous experimental investigations that were concerned with the effects of weightlessness on precellular and unicellular forms shows that viability, genetic processes, morphology and functional parameters of vital activity of the most primitive procaryote to the amoeba, as a rule, remain essentially unchanged under the

influence of the gravitational factor (or weightlessness). The findings that present an exception from this rule in most cases may be accounted for by factors other than gravitational effects.

A REVIEW OF EXPERIMENTAL WORK

Phages and viruses. Precellular biological systems at the boundary between the animate and inanimate, were studied closely to detect changes induced by space flight. The objectives of the study were on the one hand, to determine the threshold of resistance of phages and viruses to space flight effects and, on the other, to investigate possible genetic effects induced by these factors. The first experiments with phage particles were carried out on board Soviet spaceships (1,2). They allowed the conclusion that there was no statistically significant difference in the number of viable phage particles in the experimental (flight) and control samples. Systematic investigations to estimate survival of phages and viruses in a space environment were performed by American scientists (3,4,5,6). All samples protected from the direct effect of outer space survived the exposure.

Bacteria. To study the effects of weightlessness as well as weightlessness combined with ionizing radiations, bacterial suspensions in saline were flown on board the first Soviet spaceships. The data obtained showed no appreciable difference in the concentration of bacterial cells in the flight and ground-based (control) specimens (7,8).

We also carried out many experiments using cells of Bacillus brevis (9,10). The results were analyzed in terms of their theoretical and practical significance, as well as for their further applications. The data allow the general conclusion that space flight factors other than radiation, including weightlessness, do not cause appreciable changes in spontaneous and induced mutagenesis, do not influence bacterial growth, development and survival, and do not exert a modifying influence on the radiation effect. American researchers investigated an impact of weightlessness and radiation using a suspension of Escherichia Coli flown on Biosatellite-II and Gemini-11 (11,12,13) and showed that both factors did not affect bacteria.

Detailed studies of the effect of space flight factors on bacterial cells were carried out, employing Proteus vulgaris. With the aid of specially devised instruments, the culture was actively maintained throughout the flight. Postflight examination revealed, in some cases, stimulation of biomass productivity (14,15), variation in the shape and size of cells, and change of the ultrastructural organization of cells exposed to weightlessness.

Yeast. Experiments with Saccharomyces cerevisiae were conducted aboard Biosatellite-II (16). It was shown that yeast survival was not affected by weightlessness. Experimental studies of Saccharomyces ellipsoideus were also carried out on board the Soviet spaceships (17,18), with the same result.

Chlorella. Various studies were conducted with Chlorella on Earth satellites and spaceships (19,20,21,22,23,24). No significant differences in cell survival, mutation frequency and spore formation dynamics were found between experimental and control data. Similar results were obtained concerning Chlorella cellular and subcellular morphology. At the same time, an increase in the rate of cell multiplication was observed when algae were exposed to weightlessness.

Infusoria. Experiments with Paramecium aurelia were carried out in conjunction with scientists from France on the orbital station "Salyut-6" using an onboard device "Cytos" (25,26). Postflight examination showed an increase in cell size and growth rate which the authors attributed to an unspecified stimulating effect of weightlessness.

Amoeba. One of the first experiments, with Pelomyxa carolinesis was conducted aboard Biosatellite-II (27,28). The influence of space flight factors on the rate of cell division, growth and multiplication, as well as on excretion and locomotor functions was studied. A portion of the material was analyzed by electron microscopy. No differences in the cell shape and size, in ultrastructure, or in the activity and distribution of enzymes were found in the postflight samples.

Fungi. The first space experiment with Physarum policephalum was conducted on board biosatellite Cosmos-1129. The plasmodium was cultivated on a special nutrient medium in the weightless state for 18.5 days. Immediately before launch, before placing the container into the biosatellite, and postflight (at the recovery site), the surface area occupied by the plasmodium was measured in all flight and control dishes. Postflight microscopic investigations made it possible to determine, with high accuracy, the rate of fungal growth in weightlessness and on Earth. It was found that the mean surface area occupied by the plasmodium growing in weightlessness is larger than on Earth (29). A decrease in the locomotor activity of the mycomycete plasmodium may be explained by a decline in the total cell metabolism (30).

Tissue culture. For several decades, cell cultures have been successfully used in vitro in many branches of experimental biology and medicine for investigating reactions of cells which are not controlled by the higher regulatory systems of the organism. Studies on an isolated tissue culture exposed to environmental effects are performed to reach at least two goals: to determine the properties of individual cells and thresholds of their stability and variability, and to gain an insight into adaptive reactions of regulatory systems functioning at the level of individual cells. This is the reason why cultures of isolated cells and tissues are widely used as a biological test object when investigating outer space as a habitat.

Soviet studies have been conducted in a specially built device of the Biotherm type that maintained a stable temperature in the cultivation container on board space vehicles (31). The first experiment was conducted on Cosmos-368 under thermally controlled conditions. The experiment was carried out on cells isolated from the Syrian hamster (32). Postflight examination showed that the cytogenetic parameters: karyotype, rate and pattern of chromosomal aberrations in the flight cultures did not differ from the controls. The experiment was repeated on board Cosmos-782 using a tissue culture with similar parameters and allowed the conclusion that weightlessness causes genetic changes that may be detected by light microscopy (33). An experiment on hamster tissue culture carried out by the authors in cooperation with scientists from GDR on board the orbital station Salyut-6 showed that the culture's growth in weightlessness was accompanied by the formation of a large number of cell aggregates (spheroids). The flight culture also displayed fewer microcolonies and a lower density population. With respect to other parameters, the flight culture did not differ from the control. The flight cells also exhibited a higher mitotic index and larger nuclei (34).

American cytologists conducted an interesting experiment, using the culture of human embryo lung cells of the Wi-38 line on board the orbital station Skylab-3 (35). They employed a specially devised apparatus "Woodlon" to maintain a stable temperature of 37°C, to regularly change the cultivation medium, to temporally fix cytological specimens of the culture, and to perform time lapse photography. A postflight analysis showed that in the flight culture the parameters characterizing the rate of cell division and development of abnormal mitoses did not differ significantly from the controls. Electron microscopic and scanning electron microscopic examination did not reveal any differences between the flight and control cultures.

Plant cell culture. Experiments with cultures of carrot totipotent cells gave convincing evidence that the genetic program of the cell can be completely realized in the absence of gravity. The experiments were prepared by American researchers (36) and by a group of Soviet investigators and conducted on board the biosatellite Cosmos-782 (37). The cell culture was exposed to weightlessness for 20 days. The development of free single cells in weightlessness led to the formation of embryoids that developed to the stage of early formation of cotyledons. During the flight, leaf primordia and growth zones were formed, although with slight delay, as compared to the ground-based controls. Postflight, the experimental objects were allowed to grow under illumination, yielding normal adult plants. Thus, it has been demonstrated that totipotent somatic plant cells in weightlessness may develop through the entire morphogenetic cycle and form viable embryos.

THEORETICAL ANALYSIS

In his famous publication, Pollard discusses the laws governing the distribution of molecules (large and small) in a cell of typical size (38).

Applying Pirri's equation, Pollard has convincingly demonstrated that in those cells of a volume no more than 1 mm^3 diffusion plays the major part in the distribution of substances. With increase in the cell size, convective processes become more important and, therefore, gravity may affect mass exchange (39).

As known, the largest procaryotes are no larger than 1 μm and the largest eukaryotes - 50μm. There are but few exceptions. For instance, cells of certain algae may be as long as 5 mm and those of myxomycete fungi even longer. The morphofunctional peculiarities of those latter in relation to gravity will be discussed below.

It appears that in order to guarantee life, evolution needed to spatially delimit the size of its major unit, the cell. In order to function normally, the cell must be limited in size because of two reasons. First of all, it is necessary so that diffusion can supply every cellular area with nutrient substances with equal probability, since the maximal distance for efficient diffusion is no more than 1 mm (40). Another factor limiting cell dimensions is the fact that the ratio of the cell volume to its surface must be roughly constant and equal to about 1:3 or less. If the ratio increases, diffusion fails to maintain reliable transport in the cell (41). Therefore, to provide effective diffusion the cell should be of such shape and size as not to allow any point within it to be located more than 1 mm from the surface.

The effect of gravity (or weightlessness) can be real if its polar influence is more efficient than the equiprobable diffusion in every direction. This possibility is ruled out for the cell whose volume is no more than 1 mm^3. Therefore, all unicellular free-living organisms are gravity-independent. This implies that a change in the magnitude or direction of gravity, including its nullification (zero-g or weightlessness) cannot influence these organisms. The results of many studies of microorganisms flown on board space vehicles are in good agreement with the theoretical speculations (42). The major conclusion drawn from these experiments is that weightlessness induces no important changes in spontaneous or induced mutagenesis, growth, development or survival of unicellular organisms.

While the behaviour of individual free-living unicellular organisms in a gravity field (1 g or 0 g) is more or less clear, the peculiar features of the functioning of the unicellular population, its growth and distribution need further study by researchers working in gravitational biology.

Many unicellular populations generate, in liquid media, specific types of distribution which can be maintained over long periods of time by gravity-dependent bioconvection (43). Because in weightlessness the convective movement is absent, it can be expected that chemical gradients can be equalized and, consequently, concentration gradients in the cell and the rate of its growth can be modified. These speculations may account for the results of certain experiments in weightlessness that revealed differences in the growth rate of cells flown on board the spacecraft and those cultivated on the Earth. It is obvious that potential effects of gravity on the cell, which is a constituent of a multicellular plant or animal organism, may be much more complicated. This involves, first of all, plant geotropisms; it can be said that orientation of major organs of plants in the gravitational field is cellularly regulated (44, 45, 46). Evidently, the geotrophic bending of plant organs is a result of active shifts of the cellular contents (starch granules statoliths, or the growth hormone auxin, for example) in response to a gravitational stimulus (47, 48). Secondly, these complications are associated with the peculiarities of early embryogenesis in animals. As known, eggs of most representatives of amphibians, fish and birds are asymmetric in their mass distribution.

Some experiments conducted in true weightlessness and others in clinostatic simulated weightlessness have demonstrated that these exposures may produce a wide spectrum of abnormalities in cell cleavage due to which early embryogenesis loses its stability. These abnormalities occur only at the earliest developmental stages between the formation of the first separating furrow, and the formation of the gastrula (49). There is however a different point of view, also based on the experimental findings, holding that the cell polarity develops long before fertilization of the ovum. We cannot yet exclude the possibility that the normal development of fertilized eggs in weightlessness requires only a short-term gravitational stimulus (50).

CONCLUSIONS

1. Unicellular free-living organisms are indifferent to alterations of the extent and the direction of gravitational field. Weightlessness does not directly affect cell metabolism, since the major biological processes taking place at subcell and molecular levels are gravitation-independent.

2. Weightlessness can alter integral indices of functioning of the suspension of unicellular organisms (the population density, growth dynamics and the release of a biomass). However, these alterations are of indirect nature and can be realized due to changes in concentration gradients within the medium.

3. An effect of weightlessness at the cellular level is possible provided the cell functions within the single multicellular organism (animal or plant). In this case, cellular changes should be regarded as a result of the shift of general metabolism at the level of a whole organism which is first of all associated with the action of this factor on gravitation-dependent regulatory systems.

References

1. Zhukov-Verezhnikov N.N., Maysky I.W., Jazdowsky V.I., Pehov A.P., Gurdzian A.A., Ribakov N.I., Antipov V.V. Problems of cosmic biology (USSR), 2, 140, (1962).

2. Zhukov-Verezhnikov N.N., Jazdowsky V.I., Maysky I.W., Tribulev A.P., Saksonov P.P., Ribakov N.I., Antipov V.V. Ibid., 3, 184, (1962).

3. Hotchin J. Lorenz P., Hemenway C. Nature, 206, 442, (1965).

4. Hotchin J., Lorenz P., Markussen A., Hemenway C. Life sciences and space research, 5, 1, (1967).

5. Lorenz P., Hotchin J., Markussen A., Orlob I., Hemenway C., Hallgren D. Space Life Sciences, 1, 118, (1968).

6. Lorenz P.,Orlob P., Hemenway C. Ibid., 1, 481, (1969).

7. Zhukov-Verezhnikov N.N., Maysky I.N., Jazdowsky V.I., Pehov A.P., Gurdzian A.A., Ribakov N.I., Antipov V.V. Microbiology (USSR), 30, 809, (1961).

8. Zhukov-Verezhnikov N.N. Volkov M.N., Ribakov N.I., Maysky I.N., Saksonov P.P., Guberniev M.A. Cosmic research (USSR), 9, 282, (1971).

9. Parfyonov G.P. Lukin A.A. Space Life Sciences, 4, 139, (1973).

10. Parfyonov G.P., in: The basis of cosmic biology and medicine. Moscow, Nauka, 2, 406, (1975).

11. Mattoni R. Radiation Res., 35, 2, 488, (1968).

12. Mattoni R. Keller S., Ebersold W., Eiserling F.,Romig W., in: The Experiments of Biosatellite-2, NASA, Washington, 309, (1971).

13. Deserres T. The Physiologist, 16, 2, pp.225, (1973).

14. Kordium V.A., Polivoda A.V., Mashinsky A.L.,in: The Gravity and the Organism, ser. The Problems of Cosmic Biology, 33, Moscow, Nauka, 238,(1976).

15. Kordium V.A., Polivoda A.V., Mashinsky A.L., Manko V.G., Wechitailo G.S. Konshin W.I., Gavrish G.G., in: Experimental Research in Cosmic Biology, Pushino, USSR, 108, (1976).

16. Borstel von R.S., Smith R.H., Whitting A.R., Grach D.S., in: The Experiments of Biosatellite-2, Washington, NASA, 17, (1971).

17.Korogodin V.Yu Benivolensky V.N., Bliznik K.M., Kapulsevich Yu. G., Peshin V.G. Cosmic Biology and Medicine (USSR), 6, 10, (1971).

18. Benevolensky B.N., Kapulsevich V.G., Korogodin Y.Yu. Ibid. 6, 14,(1971)
19. Semenenko V.Ye a. Vladimirova M.T. Physiology of Plants,8,6, 743,(1961).

20. Shevchenko V.A., Sakovich I.S., Mesherykova B.N., Petrovnin G.P. Cosmic Biology and Medicine (USSR), 1, 25, (1967).

21. Galkin T.V. a. Alexandrova N.V. Ibid., 5, 39, (1971).

22. Vaulina B.N. a. Anikeeva I.D. Life sciences and space research,8,(1970)

23. Meleshko G.I., Shetlik I.V., Kordium V.A., Galkina T.B., Sichev V.A. Symposium on Cosmic Biology and Medicine Intercosmos, Varna, 47, (1981).

24. Meleshko G.I., Shetlik I.V., Kordium B.A., Dauha I.V., Galkina T.B., Polivoda A.V. 12th Symposium on Cosmic Biology and Medicine, Intercosmos, Warszawa, 31, (1979).

25. Planel H. Soleilhavai P.Y., Croute T. Life sciences and space research, 13, 173, (1975)

26. Planel H., Tixador R., Nefedov Yu., Gretchko G., Richoilley G. Ibid. 17, 139, (1978).

27. Abel Z.,Haack D.,Price R., in: The Experiments of Biosatellite-2, Washington, NASA, 291, (1971).

28. Ekberg D. Silver E., Bushay Y. Ibid. 273, (1971).

29. Tairbekov M.G., Parfyonov G.P., Platonova R.N., Abramova V.M., Golov V.K., Rostopshina A.V., Lyubtchenko V.Yu., Chuchkin V.G. Adv. Space Research, 1, Issue 14, 89 (1981)

30. Tairbekov M.G. Layrand D.B., Budnidsky A.A., Lednev V.V. Isv. Acad. Nauk USSR. ser. biol. N 5, 1982.

31. Isupak E.A. a. Jordanishvili Ye. I., in: Experimental Research in Cosmic Biophysics, Pushino, USSR, 165, (1976).

32. Sushkov F.V. Portugalov V.V., Rudneva S.V., Bobkova, Jordanishvili Ye. I., Isupak E.A. Cosmic Biology and Aviacosmic Medicine (USSR), 16, N 2, 58, (1976).

33. Sushkov F.V. Rudneva S.V., Wadtochej G.A., Polikarova S.I., Portugalov V.V. Archiv of Anat., hist., and embryol. USSR, 73, N 10, 28, (1977).

34. Sushkov F.V. Maltz V. Koop F., Petrak R., Rakov A., Rudneva S.V., Stolley P., Talkendorf E., Fender A., Yunge G. COSPAR 23 plenary meeting, Budapest, 436, (1981).

35. Montgomery P., Cook I., Reynolds R., Paul I., Haytlick P., Stock D., Schultz W. Kimsey S., Furrof R., Rogers S., Campbell P. Murrelly, in: Proceedings of the Skylab-science symposium, NASA, Document TMX 58154, 467.

36. Steward F. a. Kricorian A., in: Biological Investigations on Biosatellite Cosmos, Moscow, Nauka, 96, (1979).

37. Butenko R.G. Dmitrieva H.H., Ongko B., Basirova A.V. Ibid., 118.

38. Pollard E. J. Theoret. Biol. 8, 118, (1965).

39. Kessler J. a. Bier M. Progress austronautica and aeronautica, 50, 127, (1977).

40. Bray H.G. a. White K. Kinetics and thermodynamics in biochemistry, London, 1957.

41. Farish D. Biology the human respective, N.-Y., 1978.

42. Tairbekov M.G. a. Parfyonov G.P. Cosm. biol. and aviacosm. medicine (USSR), N 2, 51, (1981).

43. Platt J. Science, 133, 3466, (1961).

44. Heberland G.- Jahrb. Wiss. Bot. , 38, 447 (1901).

45. Cholodny N.G. Ibid. 65, 458 (1926).

46. Audus L. Physiol. plantorum 17, 437, (1964).

47. Tairbekov M.G. Maylan E.S., Rozov A.N. Dokl. Acad. Nauk USSR, 421, N 1, (1978).

48. Tairbekov M.G. a. Parfyonov G.P. Izv. Acad. Nauk USSR, Ser.biol. N 4, 535, (1978).

49. Dorfman Ya., Cherdancev V., in: Environment and Development Organism. Moscow, Nauka, 140, 1977.

50. Schaf S.R. a. Gerhart L.C. Developmental Biology, 79, 181, (1980).